A novel role for microRNA miR-218 in regulating invasiveness of endometriotic cells

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Endometriosis is an estrogen-dependent disease characterized by ectopic growth of endometrium-like glands and stroma outside the uterus. It has a considerable impact on endometrial receptivity: it is estimated, that 6%–10% of women in general and 35%–50% of women with pelvic pain or infertility suffer from the disease. Noteably, the expression of numerous microRNAs is dysregulated in endometriosis. microRNAs are small non-coding RNAs which regulate gene expression at the posttranscriptional level. Here, we characterize the functional impact of one microRNA previously show to be dysregulated in endometriosis – miR-218 – using an in vitro system.

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In the human endometriotic epithelial cell line 12Z, we are able to demonstrate that miR-218 upregulation via transient results in reduced invasiveness in matrigel invasion chamber. miR-218 reduced cell viability in immortalized ST-T1b endometrial stroma cells. qPCR analysis in 12Z cells, in ST-T1b, and in patient-derived primary endometrial stroma cells revealed a significant downregulation of two predicted targets of miR-218, the epidermal growth factor receptor EGFR, and the proteoglycan decorin. While EGFR expression has been shown to be upregulated in severe versus mild disease, decorin is known to be expressed in an estrogen-dependent manner in the endometrium.

Results

qPCR-confirmation of successful upregulation of miR-218 after transient transfection of the cell lines ST-T1b (human endometrial stroma cells) and 12Z (epithelial endometriotic cells). N>3, ***p<0.001, error bars = SEM.

Bioinformatic prediction analysis according to the microRNA.org database identifies the TGFbeta-binding proteoglycan decorin and the epidermal growth factor receptor EGFR as putative targets of miR-218. Alignment of the active ‘Seed’-region of the miRNA with the 3’UTR of the predicted targets.

qPCR analysis demonstrates consistent downregulation of EGFR by miR-218 in 12Z, ST-T1b and primary human endometrial stroma cells of endometriosis patients. Significantly reduced expression of EGFR in miR-218 transfected cells (qPCR), n>6, **p<0.01, *** p<0.001, error bars = SEM.

Modulation of signaling pathways by Decorin. Decorin has been shown to have an impact on IGFIR, ErbB, cMet, FGFR, CXCR4, EGFR and TGFbeta signaling. (Sofeu Feugaing et al., Eur J Cell Biol 2012).

Conclusion

miR-218 emerges as a novel regulator of EGFR and Decorin in endometriosis, which is potentially linked to invasive cell behaviour. Decorin contributes to the pathogenesis of malignant and inflammatory diseases by influencing angiogenesis and matrix-dependent signalling via TGFbeta and by interferon gamma signalling during inflammation. Furthermore, EGFR is involved in the regulation of cell proliferation, invasiveness and epithelial-to-mesenchymal transition, rendering these two factors promising candidates for the miR-218-dependent pathogenic mechanism.